

# **Current and Forward Looking Experimental Approaches in Gluten-Free Bread**

## **Making Research**

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## Abstract<sup>1</sup>

Research efforts on gluten-free bread making have rapidly increased during the last decade. A lot of different approaches are being used to improve the quality of these products. The techniques used in gluten-free bread making research vary widely. This review focuses on the methodological aspects of gluten-free bread making research and extracts relevant data from all Web of Science peer reviewed research articles on gluten-free bread published from 2010 to date. Recipes and methodologies are grouped by (main) starch source and list other ingredients, additives and treatments used. The focus lies on the experimental setups typically used to analyze batter/dough and end product. Small deformation rheological measurements are typically performed on gluten-free batter/dough, along with several other batter/dough properties, but there is no clear link between these characteristics and the bread quality which typically is determined by volume and texture analysis or sensory evaluation. Some more recent techniques that have already been used on wheat bread or other bakery products are discussed as well. Their application in gluten-free bread making research may help extend the current knowledge.

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<sup>1</sup> Abbreviations: <sup>1</sup>H-NMR, proton nuclear magnetic resonance; 2D, two-dimensional; ADA, acetylated distarch adipate; CLSM, confocal laser scanning microscopy; (C)MC, (carboxy)methylcellulose; DAG, diacylglycerol; DATEM, diacetyl tartaric acid ester of mono- and diglycerides; DSC, differential scanning calorimetry; ERO, electrical resistance oven; EWP, egg white powder; FOS, fructooligosaccharides; GI, glycemic index; HACS, high amylose corn starch; HDP, hydroxypropyl distarch phosphate; HPMC, hydroxypropyl methylcellulose; IR, infrared; LAB, lactic acid bacteria; LBG, locust bean gum; MAG, monoacylglycerol; ML, Mixolab; MRI, magnetic resonance imaging; RVA, rapid visco analyzer; SEM, scanning electron microscopy; SPI, soy protein isolate; SSL, sodium stearoyl lactylate; TD, time domain; TGA, thermogravimetric analysis; TGase, transglutaminase; TPA, texture profile analysis; WP, whey protein

## 1. Introduction

Gluten proteins are the main storage proteins in wheat, and are responsible for the visco-elastic properties of wheat flour dough. People who suffer from coeliac disease, an intestinal intolerance to the storage proteins of wheat (i.e. all *Triticum* species), rye and barley (and sometimes oats), need to exclude these from their diet. While consumption of pure oats is safe for most coeliac patients, it is considered a gluten containing cereal in many countries because it is often contaminated with gluten containing cereals. Not only coeliac disease patients, but also people who suffer from non-coeliac gluten sensitivity and an increasing share of consumers who avoid gluten for lifestyle reasons follow a gluten-free diet. Whether or not linked to coeliac disease or other gluten-related disorders, gluten-free diets attract a lot of attention in the media nowadays. The market of gluten-free products grows and enormous efforts are underway to enhance their quality. Literature has described the lack of cohesiveness and elasticity of gluten-free batters/doughs. They are more difficult to handle than wheat flour dough and have poor gas holding capacity. The end products have a low volume, a crumbly texture, pale color, poor flavor and firm rapidly.

## **2. Peer-reviewed literature on gluten-free bread making is limited**

The use of gluten-free ingredients such as alternative flours, starches, hydrocolloids, proteins, enzymes, lipids, pseudocereals, and sourdough have been extensively reviewed (Alvarez-Jubete et al., 2010; Anton and Artfield, 2008; Arendt et al., 2007; Capriles and Arêas, 2011, 2014; Comino et al., 2013; Deora et al., 2014; Falade and Akingbala, 2011; Gallagher et al., 2004; Gobetti et al., 2007; Houben et al., 2012; Huttner and Arendt, 2010; Kulamarva et al., 2009; Matos and Rosell, 2015; O'Shea et al., 2014; Ohimain, 2015; Omary et al., 2012; Rahaie et al., 2014; Wolter et al., 2014d; Zannini et al., 2012).

A literature search on the topic “gluten-free bread” in Web of Science in April 2015 resulted in a list of 655 articles. A critical examination of the titles and abstracts was done to exclude all papers on gluten detection, quantification and detoxification and those only describing experiments on wheat products. Figure 1 shows the distribution in time of the 399 remaining articles concerning gluten-free bread with an estimate of the number of publications in 2015 (shown in grey) based on the number of publications during its first quarter. Since 2007 more than ten research articles are published every year. A stepwise increase is seen with approximately 30 articles published in 2008 and 2009 and approximately 50 each year from 2010 to 2013. In 2014 a steep increase is seen with 70 articles published that year. This steep increase seems to continue in 2015 (based on the estimate). Capriles and Arêas (2014) noticed a similar trend after performing a literature search on “gluten-free bread” up to the end of 2013. They found a slightly smaller amount of articles on gluten-free bread, probably due to the use of stricter search criteria.

A literature search on the topic “wheat bread” performed on the same day gave approximately 4800 articles of which more than 65 % were published before 2010 while for “gluten-free bread” only 28 % of the total number of publications were from before that

66 date. A steep increase is seen in the number of articles on wheat bread from 2006  
67 (approximately 160) to 2008 (approximately 300). Since 2008 the number of publications per  
68 year on wheat bread is rather constant.

69 Considering that all research papers on wheat bread deal only with one starting material,  
70 while in the research papers on gluten-free bread over different 20 origins of flours and  
71 starches are described, the number of papers on gluten-free bread from one flour or starch  
72 source is even more limited. Furthermore, as for wheat bread, there is wide variety of bread  
73 types based on regional habits, ranging from French-style bread, Brazilian cheese bread,  
74 Middle Eastern flat bread, Indian chapatti bread to West-European and North-American  
75 sliced bread.

### 3. Methodological aspects of gluten-free bread making research

This review takes a closer look at the literature on gluten-free bread making from 2010 up to the first part of 2015, thereby covering over 70 % of all Web of Science papers on gluten-free bread making. The focus lies on the different analytical techniques used to determine the quality of gluten-free batter/dough and bread. The 399 articles included in Figure 1 were narrowed down to only those describing original research on the West-European and North-American bread types. Such bread is typically sliced before use. 132 articles remained which are incorporated in the tables below. Each table groups the publications on a particular starch source, respectively rice flour, pure starches, 'alternative flours' and a combination of different flours and starches. Table 5 lists the research on sourdough bread making. Articles that focus only on nutritional aspects such as protein or starch digestibility and glycemic index (Cornejo et al., 2015; Wolter et al., 2013, 2014a) or antioxidant capacity (Sakac et al., 2011) were not included in the tables, as well as articles focusing only on the chemical composition (Costantini et al., 2014; de la Barca et al., 2010; Krupa-Kozak et al., 2011b; Wronkowska et al., 2010).

#### 3.1 Gluten-free bread recipes

Each table lists the composition of the standard recipe, including flour or starch source(s) used and additives present both in control and tested sample. In recipes based on pure starches (Table 2), maize and potato starches are commonly combined. Some articles used only maize starch or a mix of maize and another starch. Rarely used starch sources were gluten-free wheat and rice. 'Alternative flours' (Table 3) include those from gluten-free cereal flours other than rice such as oat, corn, sorghum, millet and teff, from pseudocereals such as buckwheat, amaranth and quinoa, from tubers such as cassava and potato, from legumes such as soy and chickpea and from other raw materials such as chestnut, tigernut,

and chia. In recipes with a combination of different flours and starches as basic ingredients (Table 4), rice flour was often combined with cassava, potato or corn starch. Wheat starch, sorghum flour and flour from pseudocereals such as amaranth, buckwheat and quinoa were also used. In recipes with sourdough (Table 5), different flours, starches and combinations thereof were used.

Hydrocolloids were often used as improvers in bread based on rice flour, the most common one being hydroxypropyl methylcellulose (HPMC) but also carboxymethylcellulose (CMC) and methylcellulose (MC). A combination of guar gum and pectin was often used as additive in starch bread. Other commonly used gums were xanthan gum and locust bean gum. Pectin was also used as such or in combination with inulin or calcium citrate. Egg white, whey protein, zein and soy flour were used as protein sources. Enzymes such as transglutaminase (TGase),  $\alpha$ -amylase and maltogenic amylase were included in the standard recipe in a few cases. Other additives were psyllium fiber, maltodextrins, ascorbic acid, lecithin and emulsifiers such as diacetyl tartaric acid ester of mono- and diglycerides (DATEM) and sodium stearoyl lactylate (SSL). In recipes with alternative flours and sourdough an additive was needed less often to obtain a control bread of reasonable quality.

Variables in the experimental setup include different additional ingredients, different properties or treatments of flour or starch and variations in the bread making procedure. Variable additives were alternative gluten-free flours and starches, seeds, hydrocolloids, gums and emulsifiers, fibers, proteins and enzymes. Buckwheat, carob, quinoa, amaranth, tigernut, chickpea and yam flour were added as alternative flours. As starch sources, cassava and chestnut starch were varied in the experimental setup. Seeds from chia, strawberry and blackcurrant were also added. Hydrocolloids and gums were HPMC, gelatin, carrageenan,

alginate, tragacanth gum, locust bean gum, guar gum and xanthan gum. Fiber sources were  $\beta$ -glucan (from barley or oat), psyllium, pectin, inulin, fructans, polydextrose and fibers from bamboo, potato, pea and sugar beet. Protein sources include soy, pea and whey protein, zein, casein, egg white, collagen and bovine plasma protein. Enzymes such as TGase, different types of peptidases, glucose oxidase, laccase, xylanase, tyrosinase and amyloglucosidase were added to modify protein characteristics. Other variable additives were iron sources, apple and orange pomace, green plantain flour, acids,  $\text{NaH}_2\text{PO}_4$  and glutathione. Different varieties of rice and oat flour, rice grain sizes and flour particle sizes were tested. Treatments include extrusion, high pressure treatment and steaming or roasting of flour and the use of modified starch. The breadmaking procedure was adapted by varying the mixer type and mixing time, and baking in a conventional oven was compared to infrared microwave baking.

The water level added was very variable, ranging from 50 to 218 %. For rice bread, most recipes used a water content between 70 and 110%. In starch breads, a water content of 90-110 % was commonly used. Recipes based on alternative gluten-free flours commonly used a water content of 100 % or higher. Recipes based on a combination of flours and starches generally includes less water, ranging between 70 and 100 %. In recipes containing sourdough, usually around 95 % of water was added.

### 3.2 Analyses of batter/dough and bread

Tables 1 to 5 also list analyses performed on the batter/dough and bread, which will be discussed separately for each Table.

Table 1 lists the recipes based on **rice flour**. In 34 research articles, this was the sole starch containing ingredient. In 20 articles, properties of the batter/dough and bread were studied. Three articles only studied batter/dough properties, while in the remainder only the bread



was analyzed (see Table 1). The analyses performed on batter/dough are grouped for frequently used analyses. Rheology measurements are commonly performed on gluten-free batter/dough. Small deformation measurements are usually frequency sweep and creep-recovery tests, and were done in nine articles (Blanco et al., 2011; Demirkesen et al., 2010b; Mancebo et al., 2015; Martinez et al., 2014b; Pongjaruvat et al., 2014; Ronda et al., 2013; 2014; Torbica et al., 2010; Tsatsaragkou et al., 2014b). Large deformation measurements include extrusion tests, texture profile analysis (TPA), uniaxial extension tests and resistance to penetration. Four articles studied large deformation rheology (Buresova et al., 2014; Han et al., 2012; Ronda et al., 2013; Storck et al., 2013). Carbon dioxide production and height development during fermentation were measured by means of rheofermentometer analysis in four studies (de la Hera et al., 2013a; 2014; Gomez et al., 2013; Steffolani et al., 2014). The pasting behavior of batters/doughs during heating, by Rapid Visco Analyzer (RVA) or Mixolab analysis were studied in ten articles (see Table 1). Protein analysis was done by SDS-PAGE (Hatta et al., 2015; Kawamura-Konishi et al., 2013; Yano, 2010, 2012; Yano et al., 2013). Torbica et al. (2010) and Han et al. (2012) used differential scanning calorimetry (DSC) to assess starch gelatinization. The batter/dough structure was studied by microscopy (Kawamura-Konishi et al., 2013; Martinez et al., 2014b; Yano et al., 2013). Storck et al. (2013) measured batter/dough color.

Table 1 also lists techniques for evaluating quality aspects of rice bread. Loaf weight and volume analyses are standard and not included in the table. The main analysis of bread crumb is that of texture. Typically a two-bite-test (TPA) is used, but sometimes a single compression test is carried out. Twenty-five articles analyzed the texture of the bread crumb, of which seven monitored its evolution during storage (see Table 1). The storage time varied from one to nine days. Ronda and Roos (2011) and Hager et al. (2014) monitored

171 starch retrogradation during bread ageing by DSC analysis. Hager et al. (2014) used proton  
172 nuclear magnetic resonance ( $^1\text{H}$ -NMR) to study changes in water distribution during storage.  
173 Crumb structure has been examined by two-dimensional (2D) image analysis (Blanco et al.,  
174 2011; Furlan et al., 2015; Hager et al., 2012; Hager and Arendt, 2013; Pongjaruvat et al.,  
175 2014; Shin et al., 2010; Tsatsaragkou et al., 2012; 2014a) or micro computed tomography  
176 analysis (Demirkesen et al., 2014), and on a smaller scale by scanning electron microscopy  
177 (SEM) (Furlan et al., 2015; Hager et al., 2012; Hatta et al., 2015; Kawamura-Konishi et al.,  
178 2013; Park et al., 2014; Yano, 2010, 2012). Other common experiments were measurement  
179 of crust and/or crumb color (Furlan et al., 2015; Kawamura-Konishi et al., 2013; Park et al.,  
180 2014; Perez-Quirce et al., 2014; Pongjaruvat et al., 2014; Steffolani et al., 2014) and crumb  
181 porosity (Tsatsaragkou et al., 2014a). Because achieving an acceptable taste is often a  
182 challenge in gluten-free bread making, sensory analysis has commonly been performed on  
183 rice bread (see Table 1). Yano and coworkers (2012; 2013) used headspace gas  
184 chromatography for analyzing the odorous compounds in bread.

185 Table 2 lists 22 recipes based on **pure starches**. The variability in analyses of starch  
186 batters/doughs was less extensive than for rice batter/dough. Small deformation rheology  
187 was measured in nine out of 22 references (Aguilar et al., 2015; Juszczak et al., 2012; Korus  
188 et al., 2012; Minarro et al., 2012; van Riemsdijk et al., 2011a; 2011b; Witczak et al., 2010;  
189 2012; Ziobro et al., 2013b). Large deformation was used four times (Korus et al., 2012;  
190 Seguchi et al., 2012; van Riemsdijk et al., 2011a; 2011b). The pasting properties in  
191 batter/dough during heating as measured by RVA or Mixolab were determined by Witczak et  
192 al. (2010; 2012), Juszczak et al. (2012) and Krupa-Kozak et al. (2012; 2013).  
193 Rheofermentometer (Aguilar et al., 2015; Mariotti et al., 2013; Minarro et al., 2012) as well  
194 as DSC (Juszczak et al., 2012; Witczak et al., 2012; Wronkowska et al., 2013) analyses were

performed in three cases. Schober et al. (2010) and van Riemsdijk et al. (2011b) visualized protein structures by microscopy.

Bread crumb texture measurement was again the most common analysis method (16 out of 22 articles). Eleven of these also monitored crumb hardening during storage from one to ten days (see Table 2). In five articles, starch retrogradation was examined by DSC (Krupa et al., 2010; Witczak et al., 2010; Ziobro et al., 2012; 2013a; 2013b). Sensory evaluation was performed in six studies (Kittisuban et al., 2014; Korus et al., 2012; Krupa-Kozak et al., 2011a; 2012; Minarro et al., 2012; Ziobro et al., 2013b). Crumb structure was studied by image analysis in ten articles and visual aspects by measurement of crumb and/or crust color in 11 articles (see Table 2). Other bread analyses were those of starch and protein (Krupa et al., 2010), dietary fiber and polyphenol (Korus et al., 2012) or calcium (Krupa-Kozak et al., 2012) contents, RVA pasting behavior of dried crumb (Krupa et al., 2010), crumb porosity (Korus et al., 2012), water activity (Krupa-Kozak et al., 2013; Mariotti et al., 2013), confocal laser scanning microscopy (CLSM) and microbiological load (Minarro et al., 2012), and *in vitro* protein digestibility (Smerdel et al., 2012).

Table 3 lists 29 recipes based on '**alternative flours**'. For batter/dough, the most performed analyses were small deformation rheology (11 articles) and measurement of batter/dough properties during heating by RVA or Mixolab (ten articles, see Table 3). Large deformation rheology was performed in six papers (Buresova et al., 2014; Flander et al., 2011; Londono et al., 2015; Onyango et al., 2011b; Sciarini et al., 2010a, b), and rheofermentometer analysis in three (de la Hera et al., 2013b; Hager et al., 2012; Renzetti et al., 2010). Sciarini et al. (2010b) and Demirkesen et al. (2013a) studied starch gelatinization by DSC analysis. The batter/dough structure was visualized by SEM (Huttner et al., 2010c; Peressini et al., 2011), CLSM (Han et al., 2013) or bright field microscopy (Huttner et al., 2010c). Other analyses

performed on batter/dough (extracts) were SDS-PAGE and quantification of free amino groups (Han et al., 2013), measurement of water holding capacity (Ruhmkorf et al., 2012) and pH (Shin et al., 2013).

Twenty-three articles analysed crumb texture, and 13 of them monitored crumb hardening during bread storage (see Table 3). In four articles, starch retrogradation was monitored during bread storage by DSC (Demirkesen et al., 2013a; 2014; Hager et al., 2014; Sciarini et al., 2012a).  $^1\text{H}$ -NMR was used by Hager et al. (2014) to study changes in water distribution during storage. Hager et al. (2012) measured the water activity of bread crumb and determined the microbial shelf-life. The crumb structure was visualized in seven articles by 2D image analysis (Demirkesen et al., 2013b; 2014; Hager et al., 2012; Hager and Arendt, 2013; Ruhmkorf et al., 2012; Sciarini et al., 2010a; 2012a; Shin et al., 2013), and the crumb and/or crust color were measured in eight articles (Demirkesen et al., 2010a; 2011; 2013a; Huttner et al., 2010a, c; Kim and Yokoyama, 2011; Sciarini et al., 2010a, b). The microstructure of crumb was assessed using SEM by Hager et al. (2012) and Demirkesen et al. (2013b), CLSM by Renzetti et al. (2010) or X-ray analysis and Fourier transform infrared spectroscopy analysis by Demirkesen et al. (2014). Renzetti et al. (2010) analysed the proteins by capillary electrophoresis. Sensory analysis was performed in five studies (Demirkesen et al., 2010a; Hager et al., 2012; Kim and Yokoyama, 2011; Pasqualone et al., 2010; Shin et al., 2013).

Table 4 lists 37 recipes with a **combination of different flours and starches** as basic ingredients. Small deformation rheological measurements of batter/dough were performed in 15 articles (see Table 4). Large deformation was measured in six articles (Andersson et al., 2011; Leray et al., 2010; O'Shea et al., 2013; Sciarini et al., 2012b; Trappey et al., 2015; Velazquez et al., 2012), and the properties of batter/dough during heating by RVA or

243 Mixolab in three (Alvarez-Jubete et al., 2010; Matos and Rosell, 2013; O'Shea et al., 2013).  
 244 Rheofermentometer analysis was done in three articles (Cappa et al., 2013; Foste et al.,  
 245 2014; Martinez et al., 2014a). Five articles used DSC analysis (Crockett et al., 2011a, b; Leray  
 246 et al., 2010; Sabanis and Tzia, 2011b; Sciarini et al., 2012b). Crockett et al. (2011a, b) studied  
 247 water distribution in batter/dough by thermogravimetric analysis. The batter/dough  
 248 structure was subjected to image analysis (Cappa et al., 2013), SEM (Martinez et al., 2014a;  
 249 O'Shea et al., 2013) or CLSM (Andersson et al., 2011; O'Shea et al., 2013).  
 250 Thirty-two studies measured crumb texture, 16 of which monitored the changes during  
 251 storage from one to eight days (see Table 4). Purhagen et al. (2012) used DSC to study starch  
 252 retrogradation and <sup>1</sup>H-NMR to study changes in water distribution during storage. The  
 253 crumb structure was visualized by 2D image analysis in 14 articles and crumb or crust color  
 254 was measured in 15 references (see Table 4). Dluzewska et al. (2015) determined the crumb  
 255 porosity. Its microstructure was visualized by SEM (Alvarez-Jubete et al., 2010; O'Shea et al.,  
 256 2013; Sabanis and Tzia, 2011a, b) or CLSM (Alvarez-Jubete et al., 2010; Hager et al., 2011;  
 257 Minarro et al., 2010; O'Shea et al., 2013). Kiskini et al. (2010, 2012) measured the crust  
 258 texture. Sensory analysis was performed in 21 articles (see Table 4). Rarely used analyses  
 259 were those of chemical composition (Hager et al., 2011; Matos and Rosell, 2013;  
 260 Phimolsiripol et al., 2012; Sarawong et al., 2014), dietary fiber (Capriles and Arêas, 2013;  
 261 O'Shea et al., 2015; Phimolsiripol et al., 2012), glycemic response (Capriles and Arêas, 2013)  
 262 or microbiological load (Minarro et al., 2010).  
 263 Table 5 lists 12 recipes wherein the application of **sourdough** was compared to a control  
 264 recipe without sourdough or wherein different yeast strains were applied. Much as in Tables  
 265 1 to 4, the most common analysis on batter/dough was small deformation rheology (Capuani  
 266 et al., 2014; Falade et al., 2014; Galle et al., 2012; Huttner et al., 2010b; Moroni et al., 2011;

267 Wolter et al., 2014b, c). Further, some analyses were typical for studies with sourdough: pH  
268 of the batter/dough was measured in six articles (Capuani et al., 2014; Galle et al., 2012;  
269 Huttner et al., 2010b; Moroni et al., 2011; Novotni et al., 2012; Wolter et al., 2014c), and  
270 four articles analyzed proteins by capillary gel electrophoresis (Capuani et al., 2014; Huttner  
271 et al., 2010b; Moroni et al., 2011; Wolter et al., 2014c). Large deformation rheology was only  
272 measured in two cases (Falade et al., 2014; Hamada et al., 2013). Two papers mentioned the  
273 use of rheofermentometer analysis (Galle et al., 2012; Moroni et al., 2011) and batter/dough  
274 properties during heating (Edema et al., 2013; Hamada et al., 2013). Rarely used analyses  
275 were lactic acid bacteria determination (Huttner et al., 2010b), protein analysis by SDS-PAGE  
276 (Hamada et al., 2013), DSC and CLSM (Falade et al., 2014) and analysis of sourdough  
277 metabolites and antifungal compounds (Axel et al., 2015).

278 Ten studies analysed bread texture, and eight of them monitored the evolution of crumb  
279 texture during storage (see Table 5). Wolter et al. (2014c) and Axel et al. (2015) also assessed  
280 the microbial shelf-life. 2D image analysis was performed in six articles (Axel et al., 2015;  
281 Capuani et al., 2014; Falade et al., 2014; Rozylo et al., 2015; Wolter et al., 2014b, c), Wolter  
282 et al. (2014b) and Falade et al. (2014) also examined the microstructure of the bread crumb  
283 by SEM or CLSM, respectively. Crust and crumb color were measured by Huttner et al.  
284 (2010b), while Edema et al. (2013) assessed the crumb texture only visually. Wolter et al.  
285 (2014b, c) and Rozylo et al. (2015) analyzed the sensory properties of bread. In four articles,  
286 bread pH was determined (Galle et al., 2012; Moroni et al., 2011; Novotni et al., 2012;  
287 Rozylo et al., 2015) and Novotni et al. (2012) also measured the lactic and acetic acid  
288 concentrations. They also performed chemical analysis, measured pasting properties and the  
289 *in vivo* glycemic index.

290 Overall, the research on gluten-free bread is very divergent. The above clearly shows the  
291 variability in the composition of the recipes, the raw materials and additives used. Even  
292 within one flour source, variability is considerable. Furthermore, a conventional widely used  
293 bread making procedure [like the 'straight dough' process (Finney, 1984) for wheat bread]  
294 does not exist for gluten-free bread and as a result a broad range of water levels, mixing  
295 times and fermentation steps are used. The techniques used for analyzing batter/dough and  
296 bread also vary widely, which emerged from the above. These factors make it difficult to  
297 compare results obtained by different research groups, even within the limited amount of  
298 research dealing with the same starting materials.

## 4. Looking forward

Dough/batter properties and quality parameters of breads have been extensively assessed. However it has been difficult to draw conclusions about the ideal properties of gluten-free systems (Matos and Rosell, 2015). There thus is only scarce information about the relationships between dough/batter characteristics and bread quality. A recent study compiled data on dough/batter characteristics and bread quality from seven previous reports comprising 65 different gluten-free recipes (Matos and Rosell, 2015). Visco-elastic moduli, pasting properties (RVA) and Mixolab parameters collected from the reported gluten-free dough/batter properties were pooled and correlated with specific volume and crumb parameters of the resulting breads. Only for the reported Mixolab parameters they found strong correlations with TPA parameters of bread. TPA hardness was strongly correlated with parameters characterizing hydration during mixing (Mixolab parameter 'C<sub>1</sub>'), set-back during cooling (Mixolab parameter 'C<sub>5</sub>-C<sub>4</sub>') and end-viscosity after cooling to 50°C (Mixolab parameter 'C<sub>5</sub>'). Cohesiveness was highly negatively correlated with 'C<sub>1</sub>' [maximum torque during mixing (used to determine water absorption)] and positively correlated with 'C<sub>5</sub>-C<sub>4</sub>' [set-back during cooling to 50 °C]. Conversely, springiness was strongly positively correlated with 'C<sub>1</sub>' and negatively with 'C<sub>5</sub>-C<sub>4</sub>'. Likely due to the low number of data available from published manuscripts, no significant correlations were found between bread texture parameters and the visco-elastic moduli or the RVA pasting parameters previously reported for different gluten-free doughs/batters.

To link batter/dough parameters with bread characteristics the transition from dough/batter to bread can be studied by monitoring the changes in the batter/dough during the different processing steps. Crucial in understanding the changes is a thorough characterization of all ingredients and of the interactions between ingredients.



#### 4.1 Fermentation phase

During fermentation, the gas produced by yeast causes expansion of the gas cells previously incorporated into the batter/dough during the mixing phase (Delcour and Hoseney, 2010). Since the stability and the growth of gas bubbles generated determines the volume of the bread loaf as well as the product texture, any modification during the fermentation phase can alter the crumb structure of the product (He and Hoseney, 1991).

To improve the knowledge about the fermentation phase, dynamic monitoring of variables such as volume, density, bubble size and distribution can be very helpful. Rheofermentometer analysis to study volume increase and gas release has already been included in several studies on gluten-free bread. However, techniques aiming at imaging the batter/dough structure have not been used commonly in this field. In wheat bread dough research, in the last decade various techniques have been used to study the fermentation phase. These have included ultrasound (Elmehdi et al., 2003; Skaf et al., 2009), magnetic resonance imaging (MRI) (Bonny et al., 2004; De Guio et al., 2009; Rouille et al., 2005), CLSM (Boitte et al., 2013; Upadhyay et al., 2012), 2D imaging (Romano et al., 2013), X-ray tomography (Lampignano et al., 2013; Turbin-Orger et al., 2012) and a 3D vision system based on structured light (Verdu et al., 2015). By applying them on gluten-free batter/dough useful information about its evolution and the critical stages for gas release during fermentation phase can be obtained.

#### 4.2 Baking phase

Traditionally, bread is baked in an oven in which dough or batter is heated progressively from the outside toward the center. During baking, a temperature gradient exists from the outside to the center of the bread. The extent to which temperature-triggered reactions

347 such as protein polymerization, starch gelatinization and pasting occur in a particular part of  
348 the dough or batter during the baking process depends on the temperature–time profile  
349 experienced by that part in the baking tin. This makes the study of the changes in  
350 dough/bread during heating very difficult. This problem can be addressed using an electrical  
351 resistance oven (ERO), in which dough or batter is heated uniformly (Baker, 1939). In such  
352 oven, dough or batter placed between two electrode plates serves as an electrical resistance  
353 and heat is generated by an electric field. As a result, it is uniformly heated and the resultant  
354 bread has no crust. The heating rate can be controlled by adjusting the distance between the  
355 electrodes, the applied voltage, and the dough area in contact with the electrodes (Hayman  
356 et al., 1998; Hoseney, 1986). Wheat bread baked in an ERO using the same time-  
357 temperature profile as that in the center of a conventionally baked dough/bread shows  
358 indeed almost no temperature or moisture gradient. During storage starch retrogradation  
359 occurs to the same extent in both types of bread (Derde et al., 2014). Similar results have  
360 been obtained for cake where during conventional baking temperature gradients induce  
361 moisture gradients (Wilderjans et al., 2013). That the increase in crumb firmness of  
362 conventionally baked cakes during storage is much higher than that of ERO cakes is because  
363 crumb to crust moisture migration significantly contributes to firming of conventional cake  
364 crumb. This moisture migration occurs less in ERO cakes due to the absence of a crust (Luyts  
365 et al., 2013a). A combination of ERO and continuous oscillatory rod viscometry has been  
366 used to monitor changes in cake batter viscosity during baking. A decrease in viscosity early  
367 in heating and a subsequent rapid increase, due to starch gelatinization, was seen. The  
368 evolution of batter viscosity during the course of heating was affected in different ways by  
369 ingredients such as sugar type, shortening, egg white, hydrocolloids and emulsifiers (Shelke  
370 et al., 1990). Similar studies on gluten-free batter/dough may well help to open the “black

371 box” which the baking step presents. Knowing the impact of different ingredients on  
372 viscosity development during baking will be very helpful in understanding the effect of these  
373 ingredients on end product quality. Another way to monitor flow properties during heating  
374 is by temperature sweep rheometer tests. This technique has already been applied in gluten-  
375 free bread making research by a few authors (Falade et al., 2014; Onyango et al., 2011a;  
376 Schober et al., 2010; Vallons et al., 2010).

377 Time domain (TD)  $^1\text{H}$ -NMR is another interesting technique to open up the “black box” of  
378 the baking phase. It provides information on the molecular mobility of water and  
379 biopolymers (van Duynhoven et al., 2010) and is based on the ability of  $^1\text{H}$  to absorb energy  
380 applied by a rotating magnetic field in an external static magnetic field. The time necessary  
381 for  $^1\text{H}$  to lose the absorbed energy and return to its equilibrium state is very sensitive to  
382 molecular mobility of water and/or biopolymers in the sample (Ablett, 1992; Schmidt, 2007).

383 TD  $^1\text{H}$ -NMR detects and reflects differences in  $^1\text{H}$  mobility of water and biopolymers based  
384 on the environment the protons are in (Tang et al., 2000). This has already allowed studying  
385 water and biopolymer  $^1\text{H}$  mobility in wheat based food products including dough (Doona and  
386 Baik, 2007; Ruan et al., 1999), cake (Le Grand et al., 2007; Luyts et al., 2013b) and bread  
387 (Bosmans et al., 2013; Chen et al., 1997; Curti et al., 2011; Wang et al., 2004). Limited  
388 research on the molecular mobility of water and biopolymers in gluten-free bread was done  
389 using TD  $^1\text{H}$ -NMR (Hager et al., 2014; Purhagen et al., 2012). However, all aforementioned  
390 studies focus on the  $^1\text{H}$  mobility of starch, proteins and/or water before and after heating or  
391 during storage, but not on how this  $^1\text{H}$  mobility changes during the heating phase, which at  
392 the moment presents a “black box”. One promising way to open it is to use temperature  
393 controlled  $^1\text{H}$ -NMR (Engelsen et al., 2001) to investigate the temperature dynamics in  
394 biopolymer and water mobility during heating. This technique has been used to study

mobility dynamics of hydrated starches of other origins (rice, potatoes, peas, etc.) upon heating (Fan et al., 2013; Ritota et al., 2008; Tananuwong and Reid, 2004; Tang et al., 2001) or of more complex wheat based doughs for biscuits (Assifaoui et al., 2006) or bread (Rondeau-Mouro et al., 2015).

Furthermore, some of the imaging techniques mentioned above can be applied during heating: X-ray tomography (Babin et al., 2006) and magnetic resonance microscopy (Bajd and Sersa, 2011). By including the baking phase, the whole bread making procedure can be monitored real time from batter/dough to baked bread. By studying the impact of adaptation to the recipes during this real time monitoring, the quality determining factors of gluten-free bread can be identified.

#### 4.3 Fresh and stored bread

Various factors play critical roles in the firming (staling) of baked goods during storage. These include amylopectin recrystallization, changes in amorphous domains and interactions among other bread components (e.g., proteins and lipids). The key to these changes is distribution of water (Chen et al., 1997).  $^1\text{H}$ -NMR and MRI are two nondestructive techniques that enable detection of (changes in) the distribution and mobility of water and in the structure of the food matrix in fresh bread and during storage (Ruan et al., 1996).  $^1\text{H}$ -NMR requires only very small samples and, therefore, relates to specific portions of a bread loaf. MRI provides additional information on the spatial distribution of water content since the whole loaf of bread can be imaged and analyzed in a noninvasive and nondestructive fashion. Moisture gradients and their changes during bread storage can be surveyed. Only two references mention proton redistribution during gluten-free bread storage by  $^1\text{H}$ -NMR (Hager et al., 2014; Purhagen et al., 2012). This technique can be much further exploited for evaluating the impact of different ingredients on the molecular water dynamics in fresh

419 bread and during bread storage. Furthermore, MRI has not been applied in the field of  
420 gluten-free bread making. Information on the impact of different ingredients on the spatial  
421 (re)distribution of water in fresh bread and during bread storage can help improving the  
422 quality of gluten-free bread. For example, water proton distribution throughout wheat bread  
423 loaves supplemented with soy flour, studied with MRI, was very homogeneous in fresh  
424 breads, with minimal water migration occurring during prolonged storage. In contrast,  
425 traditional wheat bread displayed an inhomogeneous water proton population that tended  
426 to change (with higher moisture migration towards the outer perimeter of the slice) during  
427 storage (Lodi et al., 2007).

428 Taste and mouthfeel are key to bread quality. Sensory analysis has been performed in  
429 studies on gluten-free bread (Table 1-5). However, mostly only sensory analysis of fresh  
430 bread has been performed, while fast bread staling is one of the major problems in gluten-  
431 free bread making. Next to sensory analysis with a trained sensory panel, sensory data can  
432 be acquired analytically. Aroma compounds in the crust and the crumb can be identified by  
433 Aroma Extract Dilution Analysis and quantified by stable isotope dilution assays (Schieberle  
434 and Hofmann, 2012). The ratio of concentration to odor threshold provides the aroma value.  
435 Furthermore, a model mouth using a defined amount of bread mixed with human saliva and  
436 simulation of mastication (Pflaum et al., 2013) can be coupled with proton-transfer-reaction  
437 mass-spectrometry (Buettner et al., 2008; Heenan et al., 2012) to analyze the release of  
438 odorants from bread crust and crumb inline. Comparison with wheat bread can be used to  
439 steer the gluten-free recipe to approach this desired aroma profile.

440 Together with the information retrieved with traditional techniques, applying these new  
441 techniques in gluten-free bread making can reasonably be accepted to increase our

442 understanding of the structure formation, staling and sensory characteristics of gluten-free  
443 bread.

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953 **Tables**

954 Table 1: Gluten-free bread recipes based on rice flour and analyses on the batter/dough and bread.

Additive(s)	Variable additive or flour treatment	H <sub>2</sub> O	Analyses of batter/dough						Analyses of bread								Ref	
			Rheology		Rheofermento-meter	Properties during heating	SDS-PAGE	Other	Texture analysis	Storage (days)	Image analysis	Sensory analysis	DSC	Crumb/crust color	SEM	Other		
			Small deformation	Large deformation														
HPMC	rice varieties	89-144%		x				DSC	x	1								1
HPMC	rice grain length; flour particle size	80%/110%			x			SEM	x									2
HPMC	mixing time, speed dough hook/ flat beater/ wire whip	80/110%			x				x									3
HPMC	flour particle size	70-110%							x								<i>in vitro</i> starch digestibility, GI	4
HPMC	extruded rice flour		x		x	RVA		SEM	x	3								5
HPMC	chia seed	100%			x	RVA			x			x		x				6
HPMC	SPI, calcium caseinate, egg albumin, pea protein isolate acetic acid, lactic acid	80%	x			RVA												7
HPMC	acetic acid, lactic acid, citric acid, NaH <sub>2</sub> PO <sub>4</sub>	110%	x					pH			x	x						8
HPMC	-	110%							x	8			x					9
xanthan gum HPMC	-	120-130%							x		x							10
HPMC, CMC, emulsifier (sorbitan fatty acid monostearate), hemicellulase, EWP	fractions of rice flour	90%				RVA		batter color	x			x		x	x			11

improver (corn starch, SSL, ascorbic acid, $\alpha$ -amylase); xanthan gum	TGase, casein, albumin	115%		x					x								955
-	HPMC (semi-firm / weak gel forming) barley $\beta$ -glucan	70%	x	x		RVA											13
-	HPMC, barley $\beta$ -glucan	70-110%							x	9		x		x			14
-	HPMC, Psyllium fiber	90-110%	x						x								15
EWP, WPC, DATEM, LBG, $\alpha$ -amylase	carob flour	70-150%							x		x					chemical analysis, crumb porosity	16
EWP, WPC, DATEM, LBG, $\alpha$ -amylase	carob flour	70-150%	x														17
EWP, WPC, DATEM, LBG, $\alpha$ -amylase	carob flour, resistant starch	80-140%							x		x					crumb porosity	18
-	gums (xanthan/ guar/ LBG/ pectin/ HPMC) Emulsifiers (DATEM/ Purawave)	150%	x						x			x					19
-	gums (xanthan, guar, LBG, agar, MC, CMC, HPMC), emulsifier (DATEM)	143%							x							X-ray $\mu$ CT analysis	20
ascorbic acid	-	500FU		x					x								21
-	bovine plasma protein + sucrose/inulin	60%							x		x	x		x	x		22
-	peptidase	77%					x	microscopy	x						x		23
-	peptidase, thermoase	77%					x		x	3				x	x		24
TGase	WPC, sodium caseinate, SPI	110%				RVA			x		x	x					25
-	pregelatinised cassava starch, TGase	80%	x			RVA		DSC	x		x			x			26
-	buckwheat flour (husked/unhusked)	180-190%	x			ML			x			x					27
-	-	120%			x				x	5	x	x			x	water activity	28
-	-	95%							x	5			x			$^1\text{H-NMR}$	29
-	glutathione	100%				RVA	x	SEM							x		30
-	glutathione	100%				RVA	x					x			x	headspace gas analysis	31
-	glutathione	100%					x					x				headspace gas analysis	32

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961 **Abbreviations:** TPA, texture profile analysis; DSC, differential scanning calorimetry; SEM, scanning electron microscopy; HPMC, hydroxypropyl methylcellulose; GI, glycemic index; RVA, rapid  
962 visco-analyzer; (C)MC, (carboxy)methylcellulose; EWP, egg white powder; SSL, sodium stearyl lactylate; TGase, transglutaminase; WPC, whey protein concentrate; DATEM, diacetyl tartaric  
963 acid ester of mono- and diglycerides; LBG, locust bean gum; SPI, soy protein isolate; ML, Mixolab; NMR, nuclear magnetic resonance.

964 Table 2: Gluten-free bread recipes based on different types of starch and analyses on batter/dough and bread.

Standard recipe		Variable additive	H <sub>2</sub> O	Analyses of batter/dough						Analyses of bread							Ref
Starch source(s)	Additive(s)			Rheology		Rheofermentometer	Properties during heating	DSC	Other	Texture analysis	Storage (days)	Image analysis	Sensory analysis	DSC	Crumb/crust color	Other	
				Small deformation	Large deformation												
corn starch, potato starch	guar gum, pectin	maltodextrins	103%	x			RVA			x	2			x			33
corn starch, potato starch	guar gum, pectin	bean starch	100%							x	10			x		starch, protein content RVA	34
corn starch, potato starch	guar gum, pectin	defatted blackcurrant/strawberry seeds	105-118%	x	x					x	1		x		x	crumb porosity dietary fiber, polyphenol content	35
corn starch, potato starch	guar gum, pectin	ADA HDP HACS	103%	x			RVA	x		x	2	x		x			36 <i>batter</i> 37 <i>bread</i>
corn starch, potato starch	guar gum, pectin	inulin (differing in degree of polymerisation)	99-107%							x	3	x		x			38 <i>batter</i> 39 <i>bread</i>
corn starch, potato starch	guar gum, pectin	albumin lupine protein soy protein concentrate collagen pea protein isolate	105% 114% 148% 123% 128%	x						x	2	x	x	x	x		40
corn starch, potato starch	pectin	calcium caseinate, calcium citrate	97%							x			x		x		41
corn starch, potato starch	inulin, pectin	calcium citrate, calcium lactate, calcium carbonate, calcium chloride	106%				ML			x	1	x	x		x	Ca determination	42
corn starch, potato starch	pectin	buckwheat flour	80%					x		x	3	x			x		43
corn starch, potato starch	pectin, calcium citrate	calcium caseinate, sodium caseinate, WP isolate, hydrolyzed WP	105%				ML			x					x	water activity	44
corn starch	zein, HPMC	defatted zein	94%						microscopy							volume	45

corn starch	-	carob germ flour HPMC	65-84%							x							46
corn starch	xanthan gum, emulsifiers	chickpea flour carob germ flour pea protein isolate soy flour	103%	x		x				x	5		x		x	CLSM, microbiological analysis	47
corn starch, rice extrudate	HPMC, guar gum	extruded flours: buckwheat/potato flakes/corn protein source: SPI/EWP/sodium caseinate TGase	125%							x						protein digestibility	48
corn starch	<i>Psyllium</i> fiber, guar gum, maltodextrins	buckwheat, HPMC	100%			x				x	3	x			x	water activity	49
corn starch, tapioca starch, potato starch, rice flour		buckwheat, HPMC	83%														
corn starch	baking powder, xanthan gum, emulsifier	tigernut flour, chickpea flour	108%	x		x				x	4	x			x		50
wheat starch	-	WP (aggregates/cold set gel/particles)		x	x												51
wheat starch	-	WP particles	100%	x	x				CLSM			x					52
wheat starch	-	yam flour	75%		x										x		53
rice starch	-	WP isolate/ $\beta$ -glucan/ HPMC	87%							x		x	x		x		54

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969 **Abbreviations:** DSC, differential scanning calorimetry; TPA, texture profile analysis; RVA, rapid visco-analyzer; ADA, acetylated distarch adipate; HDP, hydroxypropyl distarch phosphate; HACS,  
970 high amylose corn starch; ML, Mixolab; HPMC, hydroxypropyl methylcellulose; CLSM, confocal laser scanning microscopy; SPI, soy protein isolate; EWP, egg white powder; TGase,  
971 transglutaminase; WP, whey protein.

972 Table 3: Gluten-free bread recipes based on alternative flours including gluten-free cereal flours (except rice), pseudocereals, tubers, legume  
973 flours and nuts and analyses on the batter/dough and bread.

Standard recipe		Variable additive or flour treatment	H <sub>2</sub> O	Analyses of batter/dough							Analyses of bread								Ref
Starch source(s)	Additive(s)			Rheology		Rheofermentometer	Properties during heating	DSC	SEM	Other	Texture analysis	Storage (days)	Image analysis	Sensory analysis	DSC	Crumb/crust color	SEM	Other	
				Small deformation	Large deformation														
oat flour	-	glucose oxidase laccase peptidase	89%	x		x	RVA			biochemical analyses	x						capillary electrophoresis, CLSM	55	
oat flour	-	high pressure treatment	95%	x					x	bright field microscopy	x	5				x		56	
wholegrain oat flour	-	different oat varieties peptidase	120%	x			RVA				x	3				x		57	
oat flour	HPMC	-	75-120%				RVA				x	3		x		x		58	
oat flour, potato flour	-	xylanase, laccase, tyrosinase	89%		x						x	2						59	
oat meal	-	oat β-glucan	farinograph		x					gas retention								60	
corn flour, rice flour	soy flour	-	110-218%		x		RVA	x			x	3				x		61	
corn flour, rice flour	soy flour	xanthan gum; gelatine; carrageenan; alginate; CMC	158%		x						x	3	x			x		62	
corn flour, rice flour	soy flour	CMC/xanthan gum partial baking	150%								x	3	x		x			63	
corn flour	HPMC	flour particle size	80%/ 110%	x		x	RVA			flour color	x							64	
buckwheat flour rice flour white teff flour corn flour quinoa flour sorghum flour oat flour	-	-	85% 120% 95% 90% 95% 95% 95%			x					x	5	x	x			x water activity microbial shelf-life	65	
buckwheat flour teff flour rice flour	xanthan gum HPMC	-	85-95% 95-105% 120-130%								x		x					66	

corn flour			90-100%																
oat flour rice flour	-	-	95%								x	5			x			<sup>1</sup> H-NMR	67
amaranth flour chickpea flour millet flour corn flour buckwheat flour quinoa flour	ascorbic acid	-	farinograph		x						x								68
rice flour, buckwheat flour commercial mix	-	xanthan gum/propylene glycol alginate	80-100%	x					x		x								69
rice flour, buckwheat flour	-	microbial homoexopolysaccharides HPMC	100%							water holding capacity	x	7	x						70
buckwheat flour	-	TGase, (wheat gluten)		x			ML			SDS-PAGE, CLSM, free amino group quantification	-								71
chestnut flour, rice flour	-	different ratios gums (xanthan-guar/xanthan-LBG)/emulsifier (DATEM)	150-210%	x							x			x		x			72
chestnut flour, rice flour	DATEM, guar gum, xanthan gum	different ratios rice/chestnut flour conventional/ IR microwave oven	153-203%								x					x			73
tigernut flour, rice flour	DATEM, guar gum, xanthan gum	different ratios rice/tigernut flour conventional/ IR microwave oven	150-200%				x				x				x	x			74
chestnut flour, rice flour	-	DATEM, guar gum, xanthan gum conventional/ IR microwave oven	150-186%										x				x		75
chestnut flour, rice flour	-	DATEM, guar gum, xanthan gum conventional/ IR microwave oven	150-186%								x	4			x			X-ray analysis, FT-IR analysis	76
chestnut flour	-	NaCl, sucrose, chestnut starch		x			ML												77
chestnut flour, chia flour	-	guar gum, HPMC, tragacanth gum		x			ML												78
chestnut flour	-	chia flour, HPMC, guar gum		x			ML												79
sorghum flour	-	high pressure treatment	110%	x							x	3							80
sorghum flour	EWP	cassava/ corn/ rice/ potato starch	100%		x						x	4							81
soy flour	-	raw/germinated/steamed/roasted soy flour/ HPMC	100-140%							pH, volume changes	x		x	x					82



cassava flour	-	EWP	100-120%				RVA			x			x				83
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979 **Abbreviations:** DSC, differential scanning calorimetry; SEM, scanning electron microscopy; TPA, texture profile analysis; RVA, rapid visco-analyzer; CLSM, confocal laser scanning microscopy;  
 980 HPMC, hydroxypropyl methylcellulose; CMC, carboxymethylcellulose; <sup>1</sup>H-NMR, proton nuclear magnetic resonance; TGase, transglutaminase; ML, Mixolab; LBG, locust bean gum; DATeM,  
 981 diacetyl tartaric acid ester of mono- and diglycerides; IR, infrared; GI, glycemic index; EWP, egg white powder.

982 Table 4: Gluten-free bread recipes based on a combination of flour(s) and starch(es) and analyses on the batter/dough and bread.

Standard recipe		Variable additive or flour treatment	H <sub>2</sub> O	Analyses of batter/dough							Analyses of bread							Ref	
Starch source(s)	Additive(s)			Rheology		Rheofermentometer	Properties during heating	DSC	CLSM	Other	Texture analysis	Storage (days)	Image analysis	Sensory analysis	CLSM	Crumb/crust color	SEM		Other
				Small deformation	Large deformation														
rice flour, cassava starch, corn starch	eggs, guar gum, CMC, soybean extract powder, emulsifier	-	-								x			x					84
rice flour, cassava starch	-	HPMC, xanthan	72%	x				x		TGA	x								85
rice flour, cassava starch	HPMC	SPI, EWP	optimal hydration	x				x		TGA	x			x					86
rice flour, cassava starch, soy flour	-	emulsifiers (DATEM, SSL) enzymes (glucose oxidase, α-amylase) hydrocolloids (xanthan gum, CMC, carrageenan, alginate)	65% 65% 75%	x	x			x		protein extraction	x	3d	x						87
rice flour,cassava starch	HPMC	amylopectin chain length	105%								x	3		x					88
rice flour, cassava starch	EWP, HPMC, SSL	apple pomace	115-150%	x							x	1	x			x			89
rice flour, potato starch, cassava starch, sour cassava flour	egg	sugar/sucralose/fructose/stevia/FOS/inulin	85%								x			x		x			90
rice flour, potato starch, cassava starch, sour cassava flour	egg	sucralose/fructose/stevia/FOS/inulin	85%											x					91
rice flour, potato starch	xanthan gum	amaranth/quinoa/buckwheat flour	87%					RVA			x	5	x	x	x	x	x		92
rice flour, potato starch	WPI, xanthan, HPMC	β-glucan, inulin	59-132%	x							x	5			x	x		β-glucan quantification	93
rice flour, potato starch	xanthan gum, CMC	fructans	85%								x	2		x		x		dietary fiber, glycemic response	94
rice flour, potato starch	MC	orange pomace	85-94,6%	x	x			ML + RVA		x				x	x		x		95
rice flour, potato starch	MC	orange pomace	85-100%								x	1	x					fiber analysis	96

rice flour, corn starch, potato starch	milk powder/ EWP/SPI/xanthan/ HPMC/ pectin	different blends	56-110%				ML				x			x		x		water activity chemical composition	97
corn starch, rice flour	-	HPMC	70-95%								x	6		x		x			98
corn starch, rice flour	-	HPMC, xanthan, k-carrageenan, guar gum	70-105%	x				x			x	2		x		x	x		99
corn starch, rice flour, soy flour, buckwheat flour	albumen, xanthan gum, emulsifiers (esters of MAG and DAG)	unicellular protein	90-110%								x			x	x	x		microbiological analysis	100
rice flour, corn starch, corn flour	HPMC	enzymes ( $\alpha$ -amylase/ amylglucosidase)	80%								x			x					101
quinoa flour, corn starch	HPMC	sucrose whole grain/white flour																	
rice flour, corn flour, corn starch	HPMC	quinoa flour	80%	x			x				x			x	x				102
corn starch, rice flour	HPMC	fibers (oat, bamboo, potato, pea), polydextrose	80%	x			x			SEM	x	8		x			x		103
corn flour, rice flour, rice starch, rice protein	HPMC, LBG, guar gum, maltogenic amylase	psyllium fiber, sugar beet fiber	(farinograph)				x			image analysis, farinograph	x	3		x			x		104
rice flour, corn flour, buckwheat flour, corn starch, potato starch	inulin, guar gum	freezing of dough	86%	x						temperature recording	x			x					105
rice flour, GF wheat starch	albumen, DATEM, distilled MAG, HPMC	rice bran	100% (flour basis)								x	9		x	x		x	protein, dietary fiber content	106
rice flour, GF wheat starch	albumen, DATEM, distilled MAG, HPMC	green plantain flour	120-160%								x			x			x	resistant starch content	107
corn starch, potato starch, rice flour, amaranth flour	LBG, $\alpha$ -amylase, DATEM	albumen	60-80%								x			x	x		x		108
corn flour, corn starch, potato starch, amaranth flour	HPMC, guar gum, pectin, gluconic acid lactone, LBG	freezing of dough	81%	x	x			x											109
corn starch	zein	HPMC, high $\beta$ -glucan oat bran	65-80%	x	x				x					x					110
cassava starch, corn flour	-	soybean flour, whole egg	50-58%								x				x				111
corn starch, sorghum flour	HPMC	corn/sorghum ratio	90-110			x									x				112
corn flour, corn starch, potato starch	xanthan gum	SPI, WPI, TGase	-								x	2			x			crumb porosity	113

soy flour, corn starch, amaranth flour	guar gum, lecithin, albumen, enzymes, DATEM	iron sources	80%									x			x		x		crust texture	114
soy flour, corn starch, amaranth flour	guar gum, lecithin, albumen, enzymes, DATEM	iron sources	80%									x			x		x		crust texture	115
sorghum flour, pregelatinised cassava starch	EWP	TGase	102%	x								x	4							116
sorghum flour, native/pregelatinized cassava starch	EWP	$\alpha$ -amylase	80-100%	x								x								117
sorghum flour, native/pregelatinized cassava starch	EWP	different flour/starch blends	100%	x								x	1	x						118
sorghum flour, potato starch	HPMC	different extraction rate of sorghum flour	104-135%		x							x		x						119
commercial mix (wheat starch)	guar gum, HPMC	DATEM, time-temperature-pressure treated oat flour	83-90%									x	3						DSC, $^1\text{H}$ -NMR	120

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989 **Abbreviations:** TPA, texture profile analysis; SEM, scanning electron microscopy; (C)MC, (carboxy)methylcellulose; HPMC, hydroxypropyl methylcellulose; TGA, thermogravimetric analysis;  
 990 SPI, soy protein isolate; EWP, egg white powder; DATEM, diacetyl tartaric acid ester of mono- and diglycerides; SSL, sodium stearoyl lactylate; FOS, fructooligosaccharides; RVA, rapid visco-  
 991 analyzer; WPI, whey protein isolate; ML, Mixolab; MAG, monoacylglycerol; DAG, diacylglycerol; LBG, locust bean gum; TGase, transglutaminase;  $^1\text{H}$ -NMR, proton nuclear magnetic resonance.

992 Table 5: Gluten-free bread recipes using sourdough and analyses on the batter/dough and bread.

Standard recipe		H <sub>2</sub> O	Analyses of batter/dough							Analyses of bread							Ref
Starch source(s)	Additive(s)		Rheology		Rheofermentometer	Properties during heating	pH	Capillary gel electrophoresis	other	Texture analysis	Storage (days)	Image analysis	Sensory analysis	pH	Crumb/crust color	Other	
			Small deformation	Large deformation													
oat flour	-	120%	x				x	x	identification of LAB	x	5				x		121
buckwheat flour quinoa flour sorghum flour teff flour	-	85% 95% 95% 95%	x							x	5	x	x			SEM	122
buckwheat flour bat flour quinoa flour sorghum flour teff flour	-	85% 95% 95% 95% 95%	x				x	x		x	5	x	x			microbial shelf-life	123
buckwheat flour	-	95%	x		x		x	x		x	5			x			124
buckwheat flour / brown rice flour	reducing/ oxidizing strain	100%	x				x	x		x		x					125
rice flour	-	132%		x		RVA			SDS-PAGE							volume	126
rice flour, extruded corn flour, corn starch, potato starch, buckwheat flour	inulin, guar gum, Na caseinate	110%					x			x	3			x		lactic & acetic acid concentration chemical analysis pasting characteristics <i>GI in vivo</i>	127
corn flour, rice flour, buckwheat flour	freeze-drying of sourdough	90-102%								x	3	x	x	x			128
corn flour	-		x	x					DSC, CLSM	x		x				CLSM	129
sorghum flour	-	90%	x		x		x			x	5			x			130
fonio/sorghum flour	-	75%				ML			starch analysis							crumb texture (visually)	131
quinoa flour	-	95%							analysis of sourdough metabolites & antifungal compounds	x	5	x				microbial shelf-life	132

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995 **Abbreviations:** TPA, texture profile analysis; LAB, lactic acid bacteria; SEM, scanning electron microscopy; RVA, rapid visco-analyzer; GI, glycemic index; DSC, differential scanning calorimetry;  
996 CLSM, confocal laser scanning microscopy; ML, Mixolab.

**Figure captions**

Figure 1

Literature search on the topic gluten-free bread making. Source: Web of Science, all databases, leaving out research areas in the medical field, up until April 8<sup>th</sup>, 2015. The number of publications of 2015 (shown in grey) is estimated based on the number of publications during the first three months of that year.

**Figures**

Figure 1

